Biologically Active Sesterterpenes from a New Caledonian Marine Sponge *Hyrtios sp.*†

J. Chem. Research (S), 1996, 192–193†

Marie-Lise Bourguet-Kondracki,*[a] Cécile Debitus[b] and Michèle Guyot[a]

[a]*Laboratoire de Chimie, associé au CNRS, Muséum National d'Histoire Naturelle, 62 rue Buffon, 75231-Paris Cedex 05, France*
[b]*Laboratoire de Pharmacologie, ORSTOM, B.P. A5, Nouméa, New Caledonia*

Biologically active sesterterpenes of the manoalide family, thorectolide monoacetate (**1**) co-occurring with thorectolide (**2**), were isolated from a marine sponge *Hyrtios sp.* collected in New Caledonia.

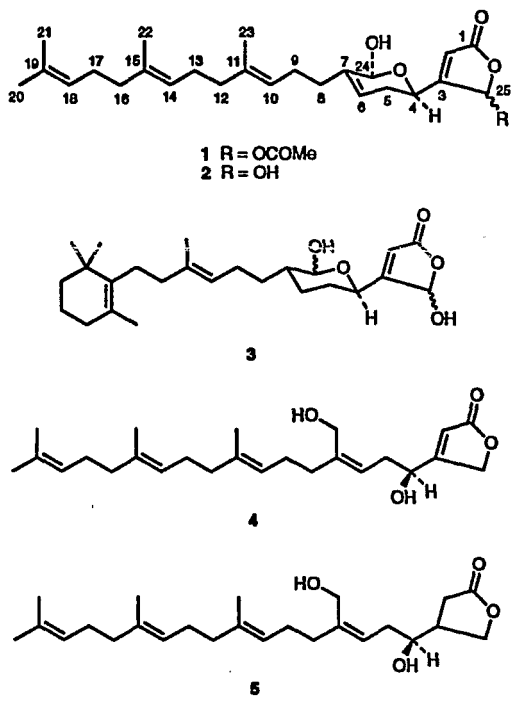
Chemical investigations of marine sponges from the genus *Hyrtios* has yielded a predominance of scalarane-type sesterterpenes.[1] We have previously isolated heteronemin and the new 12-epi-heteronemin as representatives of this class of sesterterpenes, from the marine sponge *Hyrtios erecta* collected in New Caledonia.[2] However, both scalarane-type sesterterpenes and those of the manoalide family have been clearly shown to co-occur in a sample of *Hyrtios erecta*, collected at Amani Island, Japan.[3] In our continuing chemical investigation of sponges from New Caledonia, we studied *Hyrtios sp.* which contained only sesterterpenes of the manoalide family as major constituents.

In this paper we report on the isolation and structure elucidation of thorectolide monoacetate **1** and thorectolide **2**. By HMQC and HMBC experiments we corrected the ^{13}C NMR assignment of C-5 and we assigned the resonances of carbons C-8, C-12 and C-16 of thorectolide monoacetate, previously isolated from the sponge *Thorectandra excavatus*.[4] We also investigated the stereochemistry at C-4 and C-24. Both *Hyrtios* sesterterpenes provide a further example of secondary metabolite variation within the *Hyrtios* genus.

Fractionation of the CH_2Cl_2 extract of *Hyrtios sp.*, which exhibited in a preliminary pharmacological screening significant antimicrobial activity against *S. aureus* and cytotoxic activity against KB cells, was monitored by an antimicrobial bioassay using *S. aureus*. The $CHCl_3$–MeOH 95:5 fraction, which retained maximum activity, was successively subjected to Sephadex LH-20 ($CHCl_3$–MeOH, 2:8 v/v) and silica gel (hexane–EtOAc, 7:3 v/v) column chromatography to afford thorectolide monoacetate **1** and thorectolide **2**. The known heteronemin and 12-epi-heteronemin was not detected in the CH_2Cl_2 extract of this sponge.

Compound **1** was obtained as an optically active pale yellow glass, $[\alpha]_D = +33.8°$ (*c* 0.49, $CHCl_3$). The formula $C_{27}H_{38}O_6$ determined by HREI-MS (*m/z*, 458.26684; required, *m/z* 458.26683) and the 2D NMR experiments COSY, HMQC and HMBC allowed the presence of a γ-hydroxybutenolide moiety, as in manoalide **3**[5] and luffariolides A-B[6] and F-G,[7] to be determined. This information was confirmed by a UV maximum at 212 nm (ε 4400) and by IR absorptions at 3398 and 1748 cm^{-1}. Furthermore, the EI-MS fragments at 311, 244, 137 and 69, and the ^1H and ^{13}C NMR data, suggested an isoprenoid chain of the farnesyl type. Hence, compound **1** was identical with thorectolide monoacetate.[4] However on the basis of COSY and HMQC experiments, the earlier ^{13}C NMR assignment of H-5 at δ 32.4 should be corrected to δ 28.2. HMBC experiments led us to assign the resonances of carbons C-8, C-12 and C-16 at δ 25.7, 39.5 and 39.6, respectively.

Diagnostic ^{13}C shifts at δ 15.8 for C-21, C-22 and C-23 assigned the stereochemistry of C-10–11 and C-14–15 olefins as *E*.[8] The axial nature of the H-4 protin was deduced from its coupling constants with both H-5 protons: $J = 4$ and 9 Hz. Since these H-5 protons are indistinguishable NOE experiments furnished as the only exploitable result a 10% enhancement of the OH signal at position 24 upon irradiation of the H-4 signal at δ 4.75, thus establishing an axial stereochemistry for the hydroxy group at C-24.



The absolute configuration at C-4 could be assigned unambiguously by measurement of the Cotton effect of the diol **4**, obtained by reduction of thorectolide monoacetate **1**. Reduction of **1** with $NaBH_4$[9] afforded a mixture of compounds **4** and **5**, from which the predominant diol **4** was separated by chromatography. The diol **4** displayed characteristic ^1H NMR signals at δ 4.11 (2H-24, AB system, $J = 14$ Hz), 5.39 (H-6, t, $J = 7$ Hz), 5.98 (H-2, br s), and, like the (4*R*)-manoalide diol,[10] showed a negative Cotton effect at 212 nm. Since the 4*R* absolute configuration was assigned to manoalide by comparison with both synthetic stereoisomers,[10] the absolute configuration at C-4 of thorectolide monoacetate **1** is suggested to be identical with that of manoalide.

The NMR data of **1** showed that, in contrast to the manoalide isolated from *Hyrtios erecta* collected off Amani Island,[3] only one isomer at position C-25 was present. The configuration at C-25 of thorectolide monoacetate isolated from the marine sponge *Thorectandra excavatus* was obviously the same as in **1** on account of its optical activity $[\alpha]_D = +34°$.

The new and more polar sesterterpene thorectolide **2** was obtained as an unstable colourless glass, $[\alpha]_D = +37.6°$ (*c* 0.13, $CHCl_3$) and had the molecular formula $C_{25}H_{36}O_5$. The ^1H and ^{13}C NMR spectra of **3** were almost identical with those

*To receive any correspondence.
†This is a **Short Paper** as defined in the Instructions for Authors, Section 5.0 [see *J. Chem. Research (S)*, 1996, Issue 1]; there is therefore no corresponding material in *J. Chem. Research (M)*.

of thorectolide monoacetate **1**. We noticed a missing signal assigned to the acetate methyl at δ 2.14 (20.4) and the shielding of the signal of H-25 at δ 6.15. Hence, thorectolide **2** appeared to be the desacetyl derivative of **1**. This conclusion was entirely substantiated by the HMQC and HMBC experiments (see Table 1). Hydrolysis of thorectolide monoacetate **1** yielded thorectolide **2**, identical (TLC, ^{1}H NMR and mass spectra) with the natural product **2**.

Recently, three new manoalide-related sesterterpenes co-occurring with manoalide have been isolated from the marine sponge *Fasciospongia sp.*[11] These compounds called fasciospongides A, B and C also possess an original variation of the alkyl chain attached to the γ-hydroxybutenolide moiety. In the sample of *Hyrtios sp.* studied here, manoalide was not detected.

Thorectolide monoacetate **1** exhibited the same cytotoxic activity against KB cells as did manoalide ($IC_{50} = 0.3$ μg ml^{-1}), while thorectolide **2** was much less active ($IC_{50} = 5.3$ μg ml^{-1}). Only thorectolide **2** exhibited inhibitory activity of both HIV-1 nucleocapside and integrase at 10 and 20 μg ml^{-1}, respectively. In a preliminary assay using a colorimetric method,[12] thorectolide monoacetate **1** inhibited cobra venom phospholipase A_2 up to a concentration of 2 μM but was unable to inactivate bee venom phospholipase A_2. Half-inhibition of cobra venom phospholipase A_2 was obtained for a manoalide[13] concentration of *ca.* 1.7 μM. Further studies of the potent antiinflammatory activity of these compounds are in progress.

Experimental

NMR spectra were measured on a Bruker WM300 instrument and IR spectra on a Nicolet 400D spectrometer. UV spectra were determined on a Uvikon 930 Kontron spectrometer, optical rotations on a Perkin Elmer 141 polarimeter and circular dichroism spectra on a Jobin-Yvon Mark V dichrograph. Low-resolution mass spectra were recorded on a Thomson-Houston THM 208 mass spectrometer. High-resolution mass spectra were supplied by a Kratos MS50 spectrophotometer.

Collection, Extraction and Purification.—Hyrtios sp., lyophilized sponges (500 g), collected by scuba diving in New Caledonia (Walpole), were immersed in CH_2Cl_2 at room temperature for 2 days. Solvent was removed *in vacuo* and the residue partitioned between CH_2Cl_2 and H_2O. Fractionation of the CH_2Cl_2 extract (7.7 g) was performed by chromatography on a silica gel column, eluted with $CHCl_3$–MeOH using a step gradient of increasing MeOH. Fractions were screened for antimicrobial activity using *S. aureus*. The $CHCl_3$–MeOH 95:5 (v/v) fraction, which retained maximum activity, was successively subjected to Sephadex LH-20 ($CHCl_3$–MeOH, 2:8 v/v), and silica gel (hexane–EtOAc, 7:3 v/v) column chromatography to afford thorectolide monoacetate **1** (0.004% dry weight) and thorectolide **2** (0.0006% dry weight).

Thorectolide Monoacetate **1**.—Pale yellow oil, $[\alpha]^{22}_D = +33.8°$ (*c* 0.49, $CHCl_3$) (Found: $[M]^+$, 458.26684, $C_{27}H_{38}O_6$ requires M_r 458.266834, δ_H (330.13 MHz, $CDCl_3$) 6.08 (d, J=1 Hz, H-2), 4.76 (ddd, J=4, 9, 1 Hz, H-4), 2.29 (H-5), 5.67 (br s, H-6), 2.10 (H-8), 2.12 (H-9), 5.06 (m, H-10*), 1.95 (m, H-12*), 2.05 (H-13*), 5.06 (m, H-14*), 1.95 (m, H-16*), 2.05 (H-17*), 5.06 (m, H-18), 1.64 (H-20), 1.57 (3 CH_3-21-22-23), 5.27 (s, H-24), 7.05 (s, H-25), 2.14 (H-27); δ_C (75.45 MHz, $CDCl_3$) 169.3 (C-1), 118.5 (C-2), 165.6 (C-3), 61.5 (C-4), 28.2 (C-5), 120.2 (C-6), 137.3 (C-7), 31.7 (C-8), 25.7 (C-9), 123.1 (C-10*), 134.7 (C-11), 39.7 (C-12*), 26.5 (C-13*), 123.8 (C-14*), 135.6 (C-15), 39.6 (C-16*), 26.4 (C-17*), 124.1 (C-18), 131.0 (C-19), 25.4 (C-20), 15.9 (C-21), 15.8 (C-22, C-23), 91.3 (C-24), 92.6 (C-25), 168.9 (C-26), 20.4 (C-27) (Assignments marked * may be interchanged with closest values).

Thorectolide **2**.—Colourless oil, $[\alpha]^{22}_D = +37.6°$ (*c* 0.13, $CHCl_3$), $C_{25}H_{36}O_5$; *m/z* (%) 416 (10, M^+), 398 (48), 380 (10), 355 (24), 329 (24), 283 (24), 269 (31), 247 (33), 229 (40), 215 (97), 203 (69), 137 (36), 81 (60), 69 (100); ν_{max}/cm^{-1} (KBr) 3434, 1745, 1664, 1117, 604; for δ_H and δ_C see Table 1.

$NaBH_4$ *Reduction of* **1**.—A solution of **1** (5 mg) in EtOH (3 ml) was reduced with $NaBH_4$ (2 mg) by stirring for 1 h at 0 °C. Excess reagent was destroyed by dropwise addition of 2% HCl until hydrogen evolution had ceased. The product was partitioned between brine and ether. The washed and dried extract was evaporated and the residue, a mixture of compounds **4** and **5**, purified by silica gel column chromatography ($CHCl_3$–acetone 9:1 v/v) to afford 1.4 mg of diol **4**. Oil, δ_H ($CDCl_3$) (1.59 (3 CH_3, s), 1.63 (CH_3, s), 4.11 (2H-24, AB syst., J = 14 Hz), 5.39 (H-6, t, J = 7 Hz), 5.98 (H_2, br s); *m/z* δ_H ($CDCl_3$) 1.59 (3 CH_3, s), 1.63 (CH_3, s), 4.11 (2H-24, AB syst., J = 14 Hz), 5.39 (H-6, t, J = 7 Hz)), 5.98 (H_2, br s); *m/z* (%) 402 (1, M^+), 358 (12, M−44), 137 (100).

Hydrolysis of Thorectolide Monoacetate **1**.—Excess Na_2CO_3 was added in small portions to a stirred solution of **1** (3 mg) in MeOH (2 ml) at room temperature. The mixture was stirred for 30 min, the reaction solution was extracted with $CHCl_3$ (5 ml), washed and dried over anhydrous Na_2SO_4, filtered and evaporated *in vacuo*. The product was purified by preparative TLC on silica gel (1 mm, 20 × 20 cm) to provide compound **2** (1 mg, $R_F = 0.58$ in hexane–EtOAc 7:3 v/v), δ_H see Table 1.

Table 1 NMR data for thorectolide **2** ($CDCl_3$, δ/ppm: ^{1}H, 330 MHz; ^{13}C, 75 MHz)

Atom no.	δ_C	δ_H*	HMBC correlations
1	173.8		
2	117.6	6.07 (s)	C-1, C-3
3	170.6		
4	62.6	4.84 (dd, J = 4, 9)	
5	26.5	2.11	
6	120.6	5.63 (br s)	C-24
7	137.1		
8	32.3	2.15	
9	25.8	2.12	C-8
10*	123.2	5.07 (m)	C-12, C-23
11	134.8		
12	39.4	1.95 (m)	C-10, C-11
13*	27.7	1.98	
14*	123.9	5.07 (m)	C-13, C-22
15	134.8		
16	39.4	1.95 (m)	C-14, C-15
17*	27.5	1.98	
18	124.2	5.07 (m)	C-16, C-17, C-20
19	131.0		
20	25.4	1.65	C-18, C-19
21	15.8	1.59	
22	15.8	1.59	
23	15.8	1.59	
24	91.3	5.27 (s)	C-4, C-6, C-7
25	92.6	6.15 (s)	

*May be interchanged with closest values. *a* *J* values in Hz.

We are grateful to Dr. J. Bolard for allowing us to use the Jobin-Yvon Mark V dichrograph. We thank Rhône-Poulenc-Rorer for biological assays on HIV-1, Mme C. Tempête (I.C.S.N., CNRS, Gif-sur-Yvette) for the bioassays on KB cells and Mme A. Longeon for determination of phospholipase A_2 activity. We also thank the E.E.C. for financial support (contract MAS-2-CT 91-0004).

Received, 25th August 1995; Accepted, 10th November 1995
Paper F/5/07476B

References

1 P. Crews and P. Bescansa, *J. Nat. Prod.*, 1986, **49**, 1041.
2 M. L. Bourguet-Kondracki, M. T. Martin, C. Debitus and M. Guyot, *Tetrahedron Lett.*, 1994, **35**, 109.
3 M. Kobayashi, T. Okamoto, K. Hayashi, N. Yokoyama, T. Sasaki and I. Kitagawa, *Chem. Pharm. Bull.*, 1994, **42**, 265.
4 R. C. Cambie, P. A. Craw, P. R. Bergquist and P. Karuso, *J. Nat. Prod.*, 1988, **51**, 331.
5 E. Dilip de Silva and P. J. Scheuer, *Tetrahedron Lett.*, 1980, **21**, 1611.
6 M. Tsuda, H. Shigemori, M. Ishibashi, T. Sasaki and J. Kobayashi, *J. Org. Chem.*, 1992, **57**, 3503.
7 J. Kobayashi, C. M. Zeng and M. Ishibashi, *J. Nat. Prod.*, 1993, **56**, 436.
8 P. A. Courperus, A. D. H. Clague and J. P. C. M. Van Dongen, *Org. Magn. Reson.*, 1976, **8**, 426.
9 R. S. Jacobs and J. D. Faulkner, *Eur. Pat. Appl.*, EP 133,376 (Cl, C07D307/58), 20 February 1985; *US Appl.* 519,852, 3 August 1983 (15 pp).
10 V. E. Amoo, S. De Bernardo and M. Weigele, *Tetrahedron Lett.*, 1988, **29**, 2401.
11 A. Montagnac, M. Païs and C. Debitus, *J. Nat. Prod.*, 1994, **57**, 186.
12 A. Logo de Araujo and F. Radvanyi, *Toxicon*, 1987, **25**, 1181.
13 D. Lombardo and E. A. Dennis, *J. Biol. Chem.*, 1985, **260**, 7234.